ACADEMY OF HEALTH SCIENCES, U.S. ARMY
DEPARTMENT OF CLINICAL SUPPORT SERVICES
MICROBIOLOGY BRANCH

MICROBIOLOGY DIFFERENTIAL TESTS

Microscan **Interpretation**

SMML012 version 1

07/01

Learning Step / Activity 3. Interpretation of biochemicals

a. Read the panels only if the growth well is turbid. Do not read the antimicrobics if there is no growth in the growth well.

b. Read all identification substrates against a white background except for CET and the antimicrobics used for identification (Cl_4,Cf_8, P_4, K_4, To_4) which should be read against a black background.

L.S.3. MicroScan® Interpretation

c. After 18-24 hours incubation, if the glucose is a strong yellow, the organism is considered a glucose fermenter.

d. If the glucose reaction shows any orange or red, the organism is a glucose non-fermenter.

L.S.3. MicroScan® Interpretation

L.S.3. MicroScan® Interpretation

L.S.3. MicroScan® Interpretation

1) Glucose non-fermenters may be read after 18 hours of incubation if **any** of the following tests are positive:

a) combination of ARGinine and OF/Glucose or ARGinine and CETrimide

b) LYSine

c) ORNithine

L.S.3. MicroScan® Interpretation

2) If all of these reactions are negative, **record the antimicrobics** and **reincubate** the identification

biochemicals for another 24 hours.

L.S.3. MicroScan® Interpretation

e. Prior to reading the reactions, add the following reagents to the appropriate wells.

1) VP well

a) 1 drop of 40% KOH (VP1)

b) 1 drop of 5% alpha-naphthol (VP2)

c) Allow 20 minutes for the reaction to develop

L.S.3. MicroScan® Interpretation

2) TDA well - 1 drop of 10% ferric chloride

3) Indole well - 1 to 3 drops of Kovac's reagent

L.S.3. MicroScan® Interpretation

4) Nitrate well: **on glucose non-fermenters only**

a) 1 drop of 0.8% sulfanilic acid (nitrate A)

b) 1 drop of 0.5% alpha-naphthylamine (nitrate B)

c) 5 minutes for the reaction to develop

L.S.3. MicroScan® Interpretation

f. Biochemical reactions

1) Carbohydrate fermentation

a) Positive reaction

(1) Glucose - strong yellow only

(2) Sucrose, sorbitol, raffinose, rhamnose, arabinose, inositol, adonitol, and melibiose - yellow to yellow-orange

L.S.3. MicroScan® Interpretation

b) Negative reaction

(1) Glucose - orange to red

(2) Others - red

L.S.3. MicroScan® Interpretation

2) Urea

a) Positive reaction - red to magenta

b) Negative reaction - yellow, orange, or light pink

L.S.3. MicroScan® Interpretation

3) H_2S

a) Positive reaction - black precipitate or black button

b) Negative reaction - no blackening

L.S.3. MicroScan® Interpretation

4) Indole

a) Positive reaction - red after addition of reagent

b) Negative reaction - orange after addition of reagent

L.S.3. MicroScan® Interpretation

L.S.3. MicroScan® Interpretation

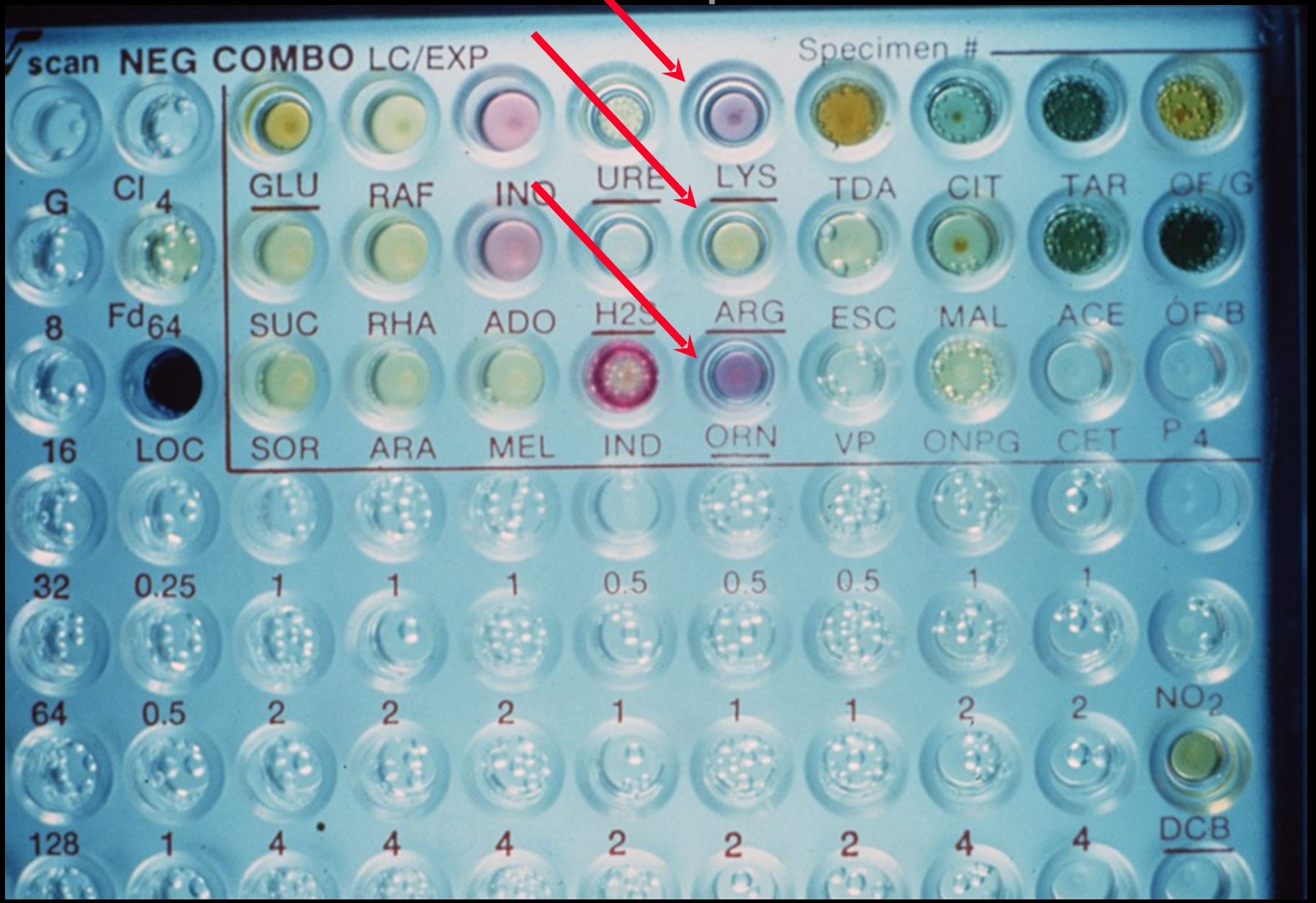
5) Lysine, Arginine, Ornithine Decarboxylases

a) Compare appearance with decarboxylase base control before interpreting results. A positive test must be more purple than the base control.

b) Positive reaction: purple

c) Negative reaction: colorless to gray/yellow

L.S.3. MicroScan® Interpretation



L.S.3. MicroScan® Interpretation

6) Tryptophane deaminase

a) Positive reaction - dark brown after addition of reagent

b) Negative reaction - yellow to orange after addition of reagent

L.S.3. MicroScan® Interpretation

7) Esculin

a) Biochemistry: The ability of any organism to hydrolyze esculin is detected by ferric ammonium citrate in the medium, which reacts with the hydrolysis products to form a black precipitate.

(1) Positive reaction: brown to black

(2) Negative reaction: clear

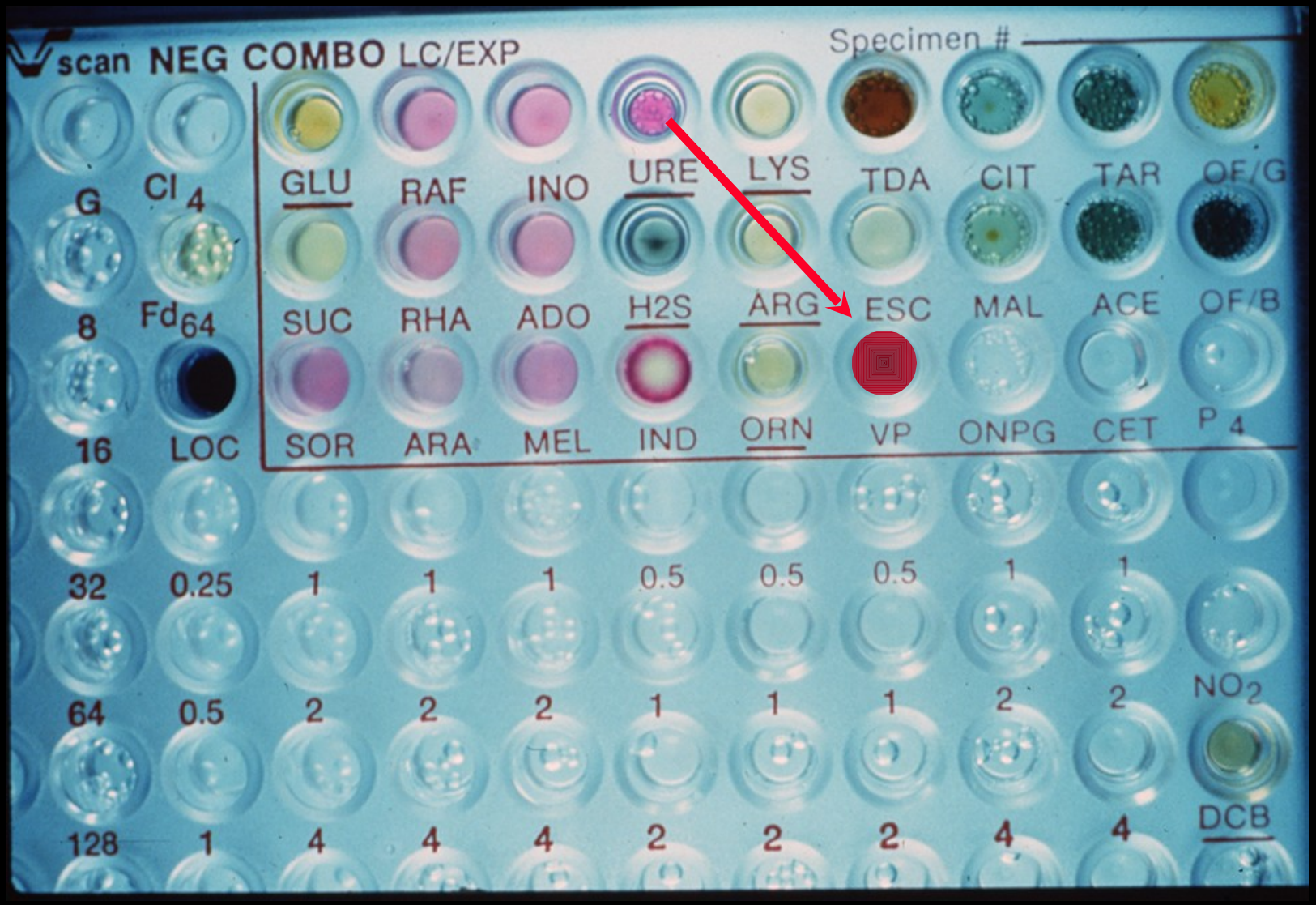
L.S.3. MicroScan® Interpretation

8) Voges-Proskauer

a) Positive reaction - red (must add reagents
and allow 20 minutes for development)

b) Negative reaction - clear

L.S.3. MicroScan® Interpretation



L.S.3. MicroScan® Interpretation

9) ONPG

a) Compare the ONPG well to the cetrimide well. Any yellow shade in the ONPG is positive when compared to the cetrimide well.

(1) Positive reaction - yellow

(2) Negative reaction - clear

L.S.3. MicroScan® Interpretation

10) Citrate, malonate, acetamide, and tartrate

a) Biochemistry - The utilization of these substrates as the sole source of carbon for metabolism results in a rise in pH, which is detected by the pH indicator bromthymol blue.

(1) Positive reaction - blue to blue-green

(2) Negative reaction - green

L.S.3. MicroScan® Interpretation

11) Cetrimide

a) Biochemistry - Tolerance to cetrimide is demonstrated by growth in Mueller-Hinton broth supplemented with cetrimide.

(1) Positive reaction - growth

(2) Negative reaction - no growth

L.S.3. MicroScan® Interpretation

L.S.3. MicroScan® Interpretation

12) Oxidation - Fermentation Carbohydrate Utilization Test

a) Purpose: this test differentiates bacteria based on the organism's ability to utilize carbohydrates either by oxidation or fermentation; or by non-utilization of carbohydrates

L.S.3. MicroScan® Interpretation

b) Principle-- Utilization of glucose in the medium,

by the bacteria results in acid products that are

detected with the bromthymol blue pH indicator,

which is also in the medium.

L.S.3. MicroScan® Interpretation

c) Interpretation uses **<u>Glu</u> AND OF/G**

(1) Fermentative (F)

i) Glu well (overlaid with oil) – Strong yellow.

ii) OF/G well (without oil) -- Yellow.

L.S.3. MicroScan® Interpretation

(2) Oxidative (OX)

i) Glu well (overlaid with oil) -- orange.

ii) OF/G well (without oil) – yellow to green

Note: Must be more yellow when compared to the OF/B. If OF/B is green then a positive reaction is yellow. If OF/B is blue then a positive reaction is yellow to green

L.S.3. MicroScan® Interpretation

13) Nitrate

a) Biochemistry: The ability of an organism to reduce nitrate to nitrite is detected by the addition of alpha-naphthylamine and sulfanilic acid, which produce a red color in the presence of nitrite. To confirm that nitrate has not been reduced to nitrogen gas, zinc powder is added to all negative tests to detect the presence of unreduced nitrate.

L.S.3. MicroScan® Interpretation

b) Nitrites, but not nitrates, form a red complex with sulfanilic acid (0.8%, Nitrate A reagent) and N,N-dimethylnaphthylamine (0.5%, Nitrate reagent).

c) The addition of zinc to a negative reaction confirms the presence of unreduced nitrates by reducing them to nitrites that react with the reagents to form a red colored complex.

L.S.3. MicroScan® Interpretation

d) No color change after the addition of zinc

indicates the complete reduction of nitrates

through nitrites to nitrogen gas, N_2, or an anaerogenic amine.

L.S.3. MicroScan® Interpretation

14) Interpretation

a) After addition of Reagents only:

(1) Positive: red color within 1-2 minutes

(2) Negative: no color change (must be confirmed)

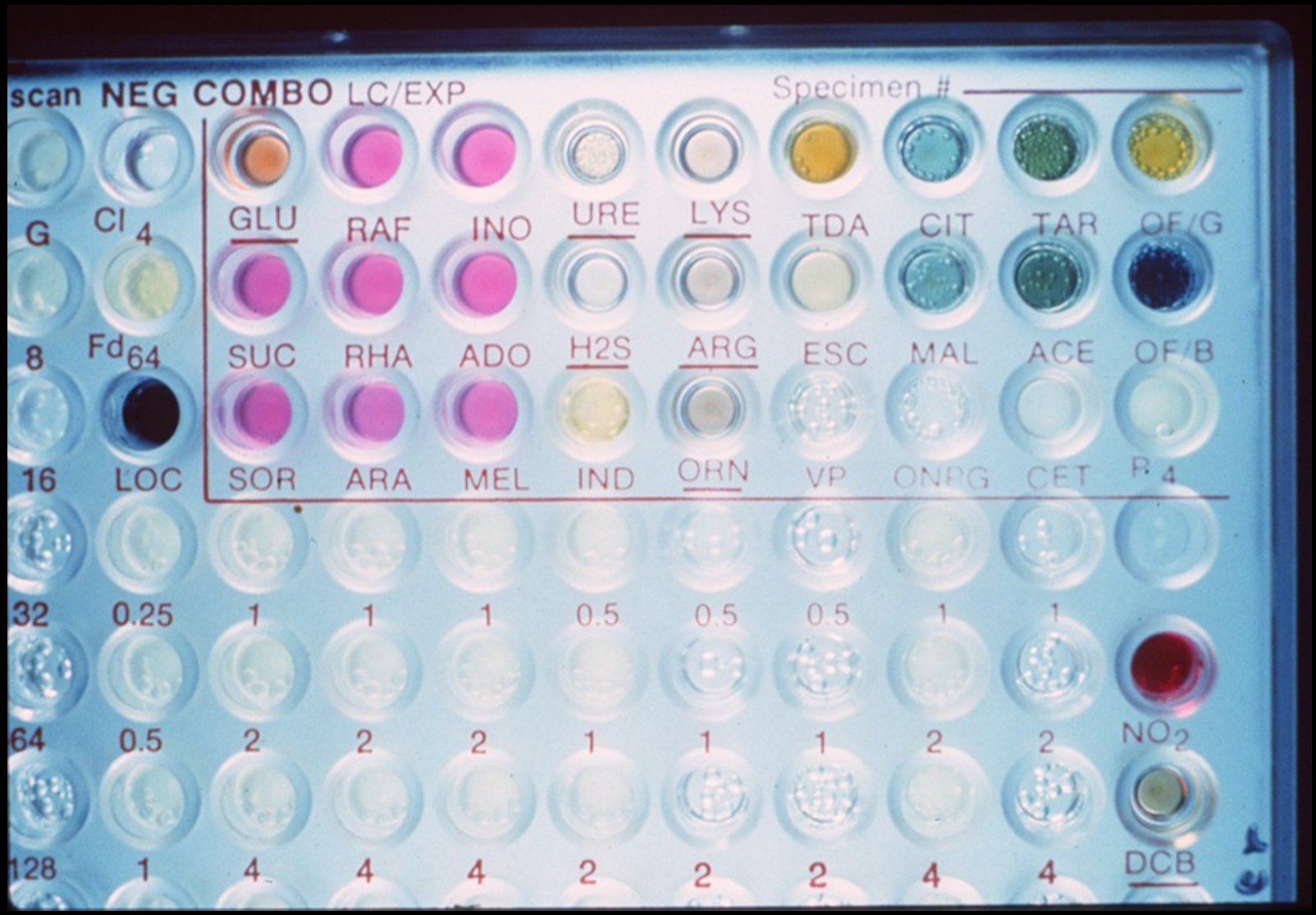
L.S.3. MicroScan® Interpretation

b) Confirm negative reactions: add small amount of zinc dust to negative test.

(1) No color change indicates reduction of nitrates to the nitrogenous state.

(2) Pink-orange color after 10 minutes confirms a negative test.

L.S.3. MicroScan® Interpretation



L.S.3. MicroScan® Interpretation

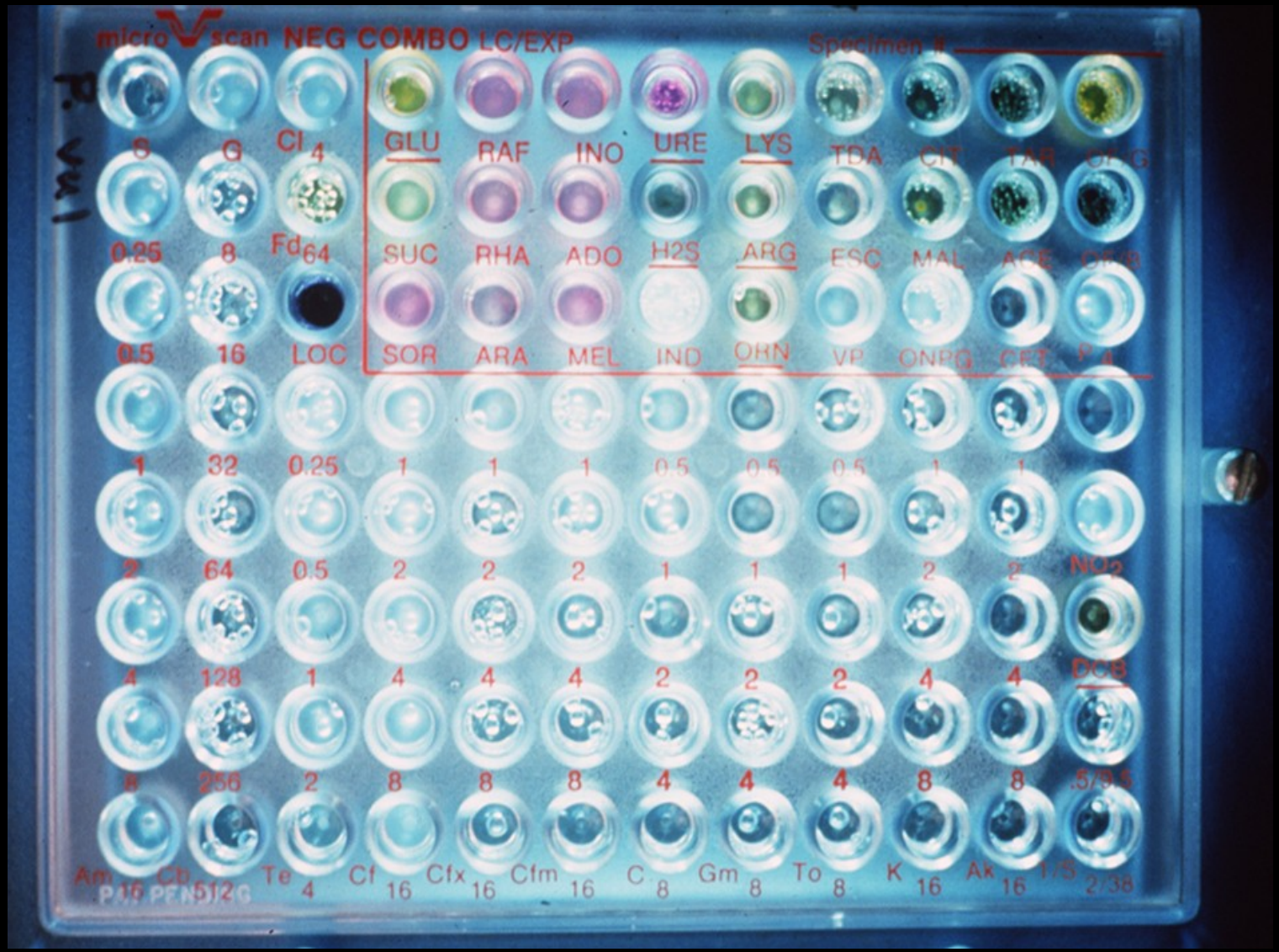
15) Penicillin, kanamycin,colistin,nitrofurantoin, tobramycin, and cephalothin

a) Biochemistry: Resistance to specific concentrations of these antimicrobial agents is demonstrated by growth

b) Positive reaction: growth (button of growth,
hazy or cloudiness)

c) Negative reaction: no growth

L.S.3. MicroScan® Interpretation



L.S.4 Identification

	Glucose Fermenter										
4	GLU	RAF	INO	URE	LYS	TDA	CIT	Cl>4	TAR	OF/G	Cl>4
2	SUC	RHA	ADO	H_2S	ARG	ESC	MAL	Cf>8	ACE	P>4	Fd>64
1	SOR	ARA	MEL	IND	ORN	VP	ONPG	OXI	CET	K>4	To>4
				Glucose Non-Fermenter							

L.S.4 Identification

a. Glucose fermenter (see 8)

1) Generation of a biotype number is obtained by assigning a weighted numerical value to all positive reactions.

a) Positive reactions in the top row get a value of 4.

b) Positive reactions in the middle row get a value of 2.

c) Positive reactions in the bottom row get a value of 1.

L.S.4 Identification

2) The biotype number is determined by adding the values for each vertical column.

3) An 8-digit biotype number is obtained and located in the biotype codebook in numerical order

a) Each number is followed by a percent probability for that organism.

L.S.4 Identification

b) Special characteristics, which are unique features of an organism, may be seen to the right of the percent probability.

c) Footnotes may be seen to help speciation.

d) The 8th digit is used only when results of colistin and cephalothin may alter interpretation,otherwise a 7-digit biotype number is printed in the codebook.

L.S.4 Identification

4) Additional tests

a) Tests which may be used to separate organisms which appear biochemically similar.

b) Listed to the right of the Footnote/ Characteristic column when the first organism is below 85% probability.

c) Up to 3 tests will be listed

L.S.4 Identification

d) Results of each test are expressed as positive percentages.

e) Examples for glucose fermenters

(1) Ox - Oxidase
(2) MR - Methyl red
(3) Mo - Motility

L.S.4 Identification

(4) Gl - Gelatin liquefaction

(5) KN- Growth in KCN broth

(6) DL - Dulcitol, Sl - Salicin,
Mt - Maltose, Xy - Xylose,
Tr - Trehalose, and
Ce - Cellobiose fermentation

(7) DN - DNase

L.S.4 Identification

b.Glucose non-fermenters

1) An 8-digit biotype number is obtained in the same manner as described for glucose fermenters.

a) A different set of tests is utilized

b) 8 digits are always printed in the biotype codebook

L.S.4 Identification

b. Glucose non-fermenters

(c) Percent probabilities follow the number

(d) Footnotes and special characteristics may follow the biotype number

L.S.4 Identification

1) Additional tests for glucose non-fermenters

(a) MC - growth on MacConkey agar

(b) Te - MIC < 4mcg/ml for Tetracycline

(c) Xy - OF Xylose (acid)

(d) Mt - OF Maltose (acid)

(e) Mnl: OF Mannitol (acid)

(f) St: starch hydrolysis

(g) DN: Dnase

(h) Le: Lecithinase

L.S.4 Identification

(i) SS: Growth on Salmonella-Shigella agar

(j) SB: Growth in 6.5% NaCl broth

(k) 42: 42° C growth

(l) Mo: Motility

(m) Lt: 10% Lactose

(n) Me: Oxidation of Melibiose

(o) Fl: Flagella

Learning Step 5. Interpretation of MIC results

a. Read against an indirectly lit background.

NOTE: The white plastic panel should be removed from the MIC test viewer.

b. Growth appears as turbidity

1) May appear as a white haze throughout the well.

2) May appear as a white button in the center of the well.

L.S.5. Interpretation

c. Growth is recorded if any whiteness can be seen in the well.

d. Criteria for interpretation

1) If the sterility control well is clear and there is turbidity in the growth well, the endpoints for antimicrobics are read.

2) If the sterility control well is not clear, contamination is suspected and the test should be repeated.

L.S.5. Interpretation

e. Lowest concentration of the antimicrobic showing complete inhibition of growth is recorded as the MIC for each antimicrobic.

1) Read from the highest concentration of antimicrobic to the lowest (usually from the bottom of the tray to the top).

2) MIC is the last clear well, not the first well in which growth appears.

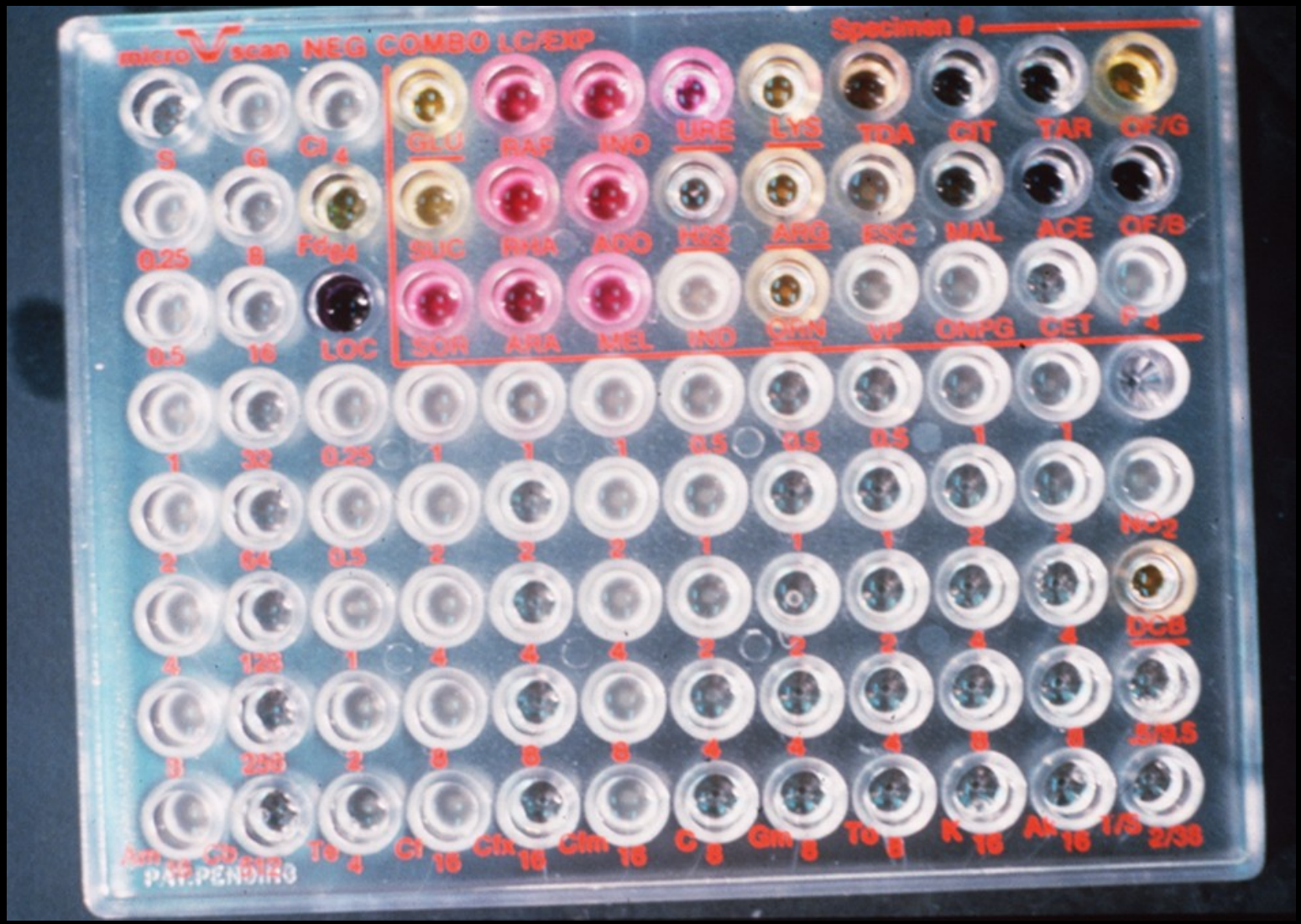
L.S.5. Interpretation

L.S.5. Interpretation

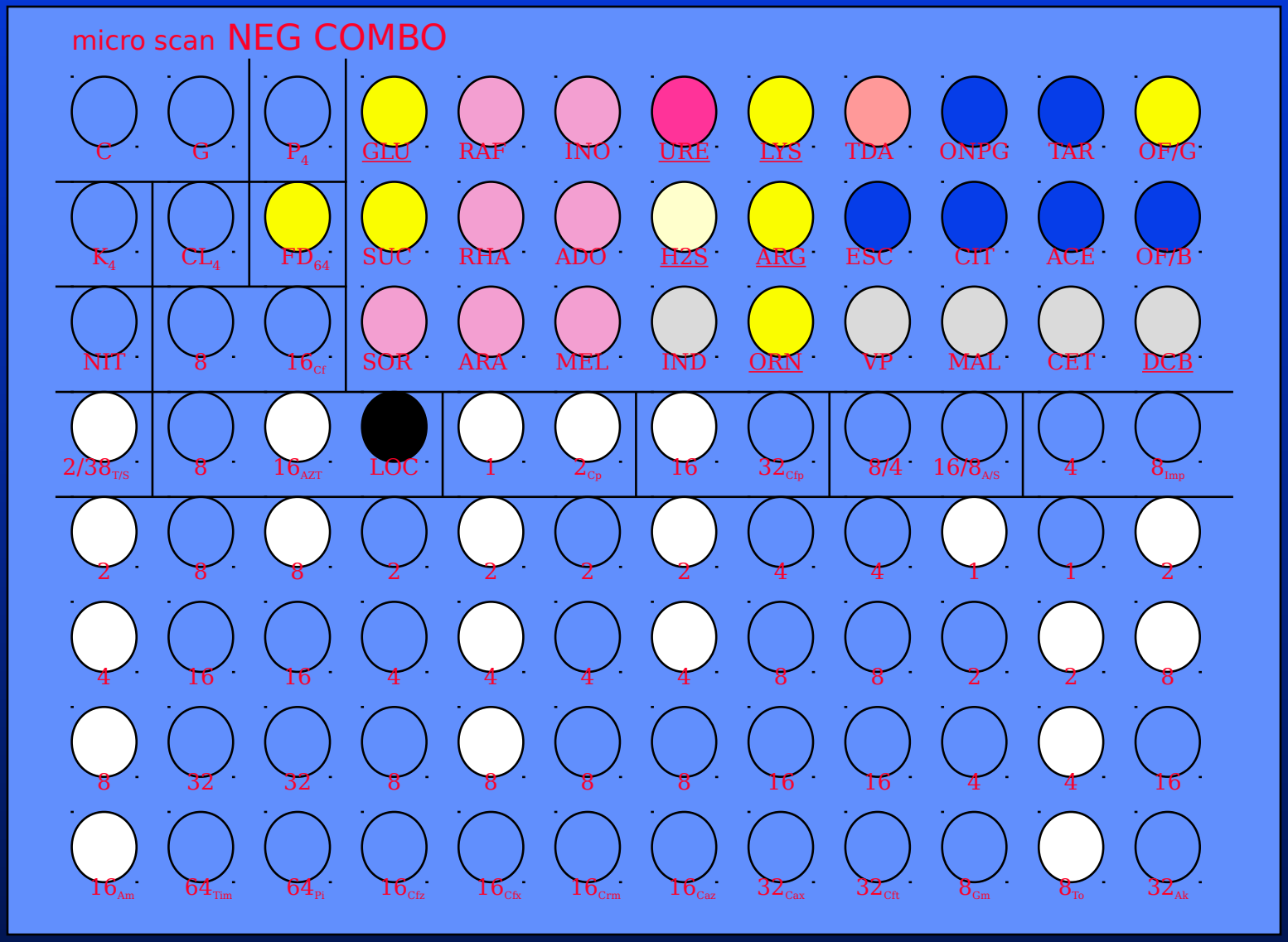
f. If all dilutions for an antimicrobic demonstrate growth, the endpoint is recorded as "greater than" the highest concentration tested.

g. If all dilutions for an antimicrobic show inhibition of growth, the endpoint for that antimicrobic is recorded as "less than or equal to" the lowest concentration tested.

L.S.5. Interpretation



L.S.5. Interpretation



L.S.5. Interpretation

a. If there is a clear well in a series of growth wells e.g., growth at 1, 2, and 8 mcg/ml, and not at

4 mcg/ml, the "skip" well should be ignored and the MIC reported as 16 mcg/ml.

L.S.5. Interpretation

i. A “trailing effect” may be observed in some drug/organism combinations such as *Proteus* with Cefuroxime (Crm) and Imipenem (Imp), *Serratia* with Imp and *E. coli* with Sulfamethoxazole (Sx). The endpoint should be read as the lowest concentration when compared to the growth well shows:

1) Approximately 80% reduction of growth (T/S, T, Sx)

2) A white button which is less than 2mm in diameter, or

3) A white button which is semi-translucent

Question: What does MIC stand for?

Answer: Minimum Inhibitory Concentration

L.S.6 Quality Control of the Microscan

a. Culture purity is checked by subculturing the final inoculum to an SBA plate.

b. Test results are invalid if a mixed culture is detected on the purity plate.

L.S.6. Quality Control

c. Testing with the following strains may check acceptability of identification media:

1) *Escherichia coli*:_ATCC 25922

2) *Pseudomonas aeruginosa*: ATCC 25922

3) *Klebsiella oxytoca*: MS #6050

4) *Proteus vulgaris*: MS #6060

L.S.6. Quality Control

d. Control organisms should produce the same MIC values, within plus or minus one serial dilution from the known values, when tested against a given antimicrobic.

e. Potency should be checked at the time of use with specific ATCC strains of *Escherichia coli* *Pseudomonas aeruginosa,* and *Klebsiella* *oxytoca.*

L.S.6. Quality Control

f. Results should meet manufacturer's specifications; deterioration of antimicrobics would result in endpoints higher than the acceptable concentrations expected

L.S.6. Quality Control

QUESTIONS??